Journal of the American College of Cardiology Vol. 49, No. 14, 2007

Published by Elsevier Inc.
ISSN 0735-1097/07/$32.00
doi:10.1016/j.jacc.2007.01.027

Telomere Length of Circulating Leukocytes Is Decreased in Patients With Chronic Heart Failure

Pim van der Harst, MD, PHD,*† Gerrit van der Steege, PHD,‡ Rudolf A. de Boer, MD, PHD,* Adriaan A. Voors, MD, PHD,* Alistair S. Hall, MD, PHD,§ Marcel J. Mulder,‡ Wiek H. van Gilst, PHD,† Dirk J. van Veldhuisen, MD, PHD, FACC,* on behalf of the MERIT-HF Study Group

Groningen, the Netherlands; and Leeds, United Kingdom

Objectives This study sought to test the hypothesis that patients with chronic heart failure (CHF) have shorter telomeres compared with age-balanced and gender-balanced healthy individuals.

Background Telomere length is considered to be a marker of biological aging. Chronic heart failure might be viewed as a condition associated with accelerated biological aging.

Methods The telomere length ratio of leukocytes was determined prospectively by a quantitative polymerase chain reaction–based method in a case-control setting involving 803 participants: 183 healthy individuals and 620 CHF patients, ages 40 to 80 years, New York Heart Association functional class II to IV, and left ventricular ejection fraction of 0.40 or less.

Results The median telomere length ratio was 0.64 (interquartile range [IQR] 0.47 to 0.88) in CHF patients compared with 1.05 (IQR 0.86 to 1.29) in control patients ($p < 0.001$). The telomere length ratio in CHF patients related to severity of disease (median value [IQR] of patients with New York Heart Association class II, III, or IV function was 0.67 [0.48 to 0.92], 0.63 [0.46 to 0.86], and 0.55 [0.46 to 0.75], respectively; p for trend <0.05). In addition, telomeres were shorter in patients with an ischemic compared with a nonischemic etiology of CHF. Patients with none, 1 (coronary, cerebral, or peripheral vascular disease), 2 (any combination of the previous), or 3 atherosclerotic manifestations had a median (IQR) telomere length of 0.72 (0.51 to 1.01), 0.65 (0.48 to 0.87), 0.48 (0.39 to 0.72), and 0.43 (0.27 to 0.67), respectively (p for trend <0.001).

Conclusions Telomere length is shorter in patients with CHF compared with age-balanced and gender-balanced control patients, and related to the severity of disease. In addition, telomere length was incrementally shorter according to the presence and extent of atherosclerotic disease manifestations. (J Am Coll Cardiol 2007;49:1459–64)

Telomeres are the distal ends of chromosomes that in humans consist of long arrays of $(TTAGGG)_n$ tandem repeat motifs that act as a cap for the ends of chromosomes. This provides protection from structural degradation, inappropriate recombination, and end-to-end fusion of chromosomes (1,2). Initial telomere length is known to be determined by genetic and environmental factors (3–7). However, at each cell division deoxyribonucleic acid (DNA) polymerases fail to completely replicate telomeres, thereby resulting in a process of cumulative erosion (8). Consequently, the structural integrity of the chromosomes becomes increasingly vulnerable with the sequential cell divisions associated with the repair processes that accompany physiological and pathological aging. When telomeres reach a critical length, cells enter replicative senescence, becoming apoptotic or otherwise genomically unstable (1,2). Telomere length thus plays a critical role in maintaining the integrity of DNA and consequently the health of cells, and can be considered to be a marker of overall biological age as compared with pure chronological age based entirely on years of life (9).

Oxidative stress, inflammation, and increased leukocyte turnover are major environmental factors associated with

From the *Department of Cardiology, †Department of Clinical Pharmacology, and ‡Medical Biomics, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; and the §British Heart Foundation Research Center, University of Leeds, Leeds, United Kingdom. This study is financially supported by Astra Hässle AB, Mölndal, Sweden, sponsor of the MERIT-HF study, by the Jan Kornelis de Cock Foundation, and by the Netherlands Heart Foundation (grant 2006B140). Dr. van der Harst is supported by Zon-MW (grant 920-03-236) of The Netherlands Organization for Health Research and Development and the Netherlands Heart Foundation (grant 2006T003). Dr. de Boer is sponsored by the Netherlands Heart Foundation (grant 2004T004). Dr. van Veldhuisen is an Established Investigator of the Netherlands Heart Foundation (grant D97-017).

Manuscript received April 4, 2006; revised manuscript received October 16, 2006, accepted November 27, 2006.

Abbreviations and Acronyms

CHF = chronic heart failure
DNA = deoxyribonucleic acid
IQR = interquartile range
PCR = polymerase chain reaction

accelerated telomere shortening and biological aging (5–7), and are also implicated in the causation of atherosclerosis. Perhaps predictably, 2 small studies have reported that telomeres were also shorter in patients with atherosclerosis (10,11), and also those with premature myocardial infarction, compared with age-balanced and gender-balanced control subjects (12).

Because chronic heart failure (CHF) is associated with increased systemic oxidative stress (13,14) and inflammation (15,16), we hypothesized that patients with CHF also would have decreased telomere length as measured for chromosomes present within circulating leukocytes. We studied telomere length in 620 CHF patients and compared our findings with those from a group of 183 age-balanced and gender-balanced healthy control patients. We aimed to investigate associations both between and within groups to assess impact of severity of CHF and also the presence of an atherosclerotic etiology.

Methods

Patients and control patients. The study was conducted in 803 participants, of whom 620 were CHF patients who participated in the MERIT-HF (Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure) study (17) who had been recruited in either the Netherlands or the United Kingdom and for whom DNA was collected (18,19). Details of the study design, inclusion and exclusion criteria, and the present subpopulation have been published previously (17–19). In brief, all Dutch and United Kingdom study participants were selected. They were ages 40 to 80 years and had had symptomatic heart failure (New York Heart Association functional class II to IV) for 3 months or more. They were receiving optimum standard therapy at enrollment and had a left-ventricular ejection fraction of 0.40 or lower measured within 3 months before enrollment. Patient characteristics and events were recorded within the framework of the MERIT-HF. For the analysis in this substudy, we used the MERIT-HF primary end point of all-cause mortality in combination with all-cause admission to hospital (time to first event).

Control subjects were a priori selected from the genetic database (n = 777) of the department of Medical Biomics of the University Medical Center Groningen, University of Groningen. This database consisted of healthy, non–blood-related relatives of patients with Crohn's disease; ages ranged from 9 to 92 years, and 45% were of male gender. Balancing for age and gender was performed a priori stratified for gender, and CHF patients were ranked for age. For the average age of 3 age-ranked CHF patients, we aimed to select 1 control subject. We were able to adequately balance 183 control subjects. We only determined telomere length in cases and the 183 selected controls. All DNA samples, MERIT-HF samples, and control samples had been stored at −80°C at the department of Medical Biomics. Determination of telomere length was performed prospectively.

We obtained ethical approval from the local regional ethics committees to perform this genetic substudy in the Dutch and United Kingdom participants. This study conforms to the principles outlined in the Declaration of Helsinki.

Telomere length assay. The DNA was collected at approximately 90 days after randomization and extracted according to standard procedures (18,19). Mean telomere length was measured from DNA by a quantitative polymerase chain reaction (PCR)-based assay (20) based on the 384-well ABI7900HT TaqMan platform (Applied Biosystems, Nieuwekerk aan de IJssel, the Netherlands). We determined the relative ratio of telomere repeat copy number (T) to single-copy gene copy number (36B4 gene, encoding ribosomal phosphoprotein PO, located on chromosome 12; S) with all samples being compared with the same reference DNA sample. The T/S ratios have been confirmed previously to be highly consistent with the classical Southern blot on terminal restriction fragments (5,21). All DNA samples were assayed in duplicate on separate plates, but in the same well positions. The mean ± SD coefficients of variation were 1.3% ± 1.2% for the T and 0.6% ± 0.6% for S assay, respectively. Determination of T and S quantities was performed using standardized thresholds and without knowledge of clinical data.

Statistical analysis. Because observed telomere lengths had a skewed distribution, the statistical analyses were performed on natural log-transformed data. Standard linear regression techniques were used to associate telomere length with individual factors and to adjust for age (in years) and gender (male or female).

One-way analysis of variance with the Scheffe post hoc test was used for multiple comparisons. For event-free survival analysis, we used the log-rank test to compare patients with a telomere length above or below the median value. In addition, we used Cox proportional hazards regression analyses to assess telomere length as a continuous variable and to also to adjust for treatment allocation, age, and gender. A 2-sided p value <0.05 was interpreted as indicating a statistical significant difference. All analyses were performed using SPSS version 12.0 software (SPSS Inc., Chicago, Illinois).

Results

Baseline characteristics of the 620 CHF patients are presented in Table 1. The CHF patients (n = 620) were adequately balanced with the healthy control patients (n = 183) with respect to mean age (66.2 ± 8.9 years vs. 66.2 ± 8.7 years, respectively) and gender (79.3% vs. 79.3% male, respectively). According to the case record forms, 183 (30%)

Table 1 Baseline Characteristics

Characteristic	Total CHF (n = 620)	Nonischemic CHF (n = 183)	Ischemic CHF (n = 437)	p Value (Nonischemic vs. Ischemic)
Male	493 (80)	127 (69)	366 (84)	<0.001
Age (yrs)	66 ± 8.7	65 ± 9.8	67 ± 8	0.013
Caucasian race	604 (97)	180 (98)	424 (97)	0.339
Current daily smoker	90 (15)	30 (16)	60 (14)	0.390
Heart failure				
Ischemic	437 (70)	0 (0)	437 (100)	
Nonischemic	183 (30)	183 (100)	0 (0)	
NYHA functional class				0.111
II	276 (45)	86 (47)	190 (44)	
III	323 (52)	95 (52)	228 (52)	
IV	21 (3)	2 (1)	19 (4)	
Previous myocardial infarction	319 (52)	0 (0)	319 (73)	<0.001
Angina	261 (42)	21 (12)	240 (55)	<0.001
Stroke	38 (6)	9 (5)	29 (7)	0.416
Claudication	68 (11)	13 (7)	55 (13)	0.046
Hypertension	206 (33)	55 (30)	151 (35)	0.278
Diabetes mellitus	97 (16)	23 (13)	74 (17)	0.172
Ejection fraction	0.27 ± 0.07	0.27 ± 0.08	0.27 ± 0.07	0.694
Heart rate (beats/min)	83 ± 10	85 ± 11	82 ± 10	0.002
Blood pressure (mm Hg)				
Systolic	132 ± 19	132 ± 18	132 ± 19	0.788
Diastolic	78 ± 9	79 ± 9	78 ± 9	0.080
Medication				
Beta-blocker	315 (51)	96 (53)	219 (50)	0.594
ACE inhibitor	545 (88)	161 (88)	384 (88)	0.970
ARB	61 (10)	18 (10)	43 (10)	0.999
Diuretics	574 (93)	173 (95)	401 (92)	0.229

Data are number (%) or mean ± SD.
ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; CHF = chronic heart failure; NYHA = New York Heart Association.

of the 620 CHF patients had CHF of nonischemic etiology. Of these 183 patients, 40 patients did in fact have other atherosclerotic disease manifestations (peripheral or cerebrovascular) or angina, leaving only 143 patients without any suggestion, signs, or symptoms of clinical manifestations of atherosclerosis.

In CHF patients, the telomere length ratio decreased steadily with age at a mean yearly rate of 0.0079 ± 0.0022 ($r = -0.143$, $p < 0.001$), which was identical in the control subjects (mean rate of 0.0077 ± 0.0026; $r = -0.220$, $p = 0.003$ and p for interaction = 0.98). Addition of squared and cubed age terms to the model had no significant effect on telomere ratio for CHF patients ($p = 0.18$ and $p = 0.28$, respectively) or for control subjects ($p = 0.75$ and $p = 0.81$, respectively). This suggests a log-linear relationship between telomere length and age.

Samples of CHF patients were collected at a mean of 90 days after randomization to placebo versus metoprolol. However, 90 days of metoprolol treatment did not affect telomere length (placebo 0.63 [IQR 0.46 to 0.86] versus metoprolol 0.65 [IQR 0.48 to 0.91], $p = 0.34$).

We observed a highly significant difference in mean telomere length ratio between CHF patients and control subjects (median 0.64 [IQR 0.47 to 0.88] vs. 1.05 [IQR 0.86 to 1.29], respectively; $p < 0.001$) (Fig. 1). In addition, the severity of the disease as indicated by New York Heart Association functional class was associated with decreased telomere length (Fig. 2A) and remained significant after adjustment for age and gender. The comparison between healthy control patients and nonischemic CHF patients validated the concept that CHF telomere length ratio was indeed shorter compared with healthy control patients ($p < 0.001$) (Fig. 2B). Furthermore, ischemic etiology of CHF was associated with shorter telomere length compared with nonischemic disease (Fig. 2B), also after adjustment for age and gender. Aside from coronary atherosclerosis, previous stroke and peripheral vascular disease were also negatively correlated with telomere length within the CHF population (Table 2). The regression coefficients of these variables remained statistically significant after adjustment for chronological age and gender (Table 2). In stepwise linear regression with forced entry of age and gender, only coronary artery disease ($p = 0.011$), stroke ($p = 0.013$), and peripheral vascular disease ($p = 0.002$) remained independently associated with telomere length. Because coronary disease, stroke, and peripheral vascular disease were independent predictors of shorter telomere length, we examined whether the coexistence of these atherosclerotic disease

JACC Vol. 49, No. 13, 2007
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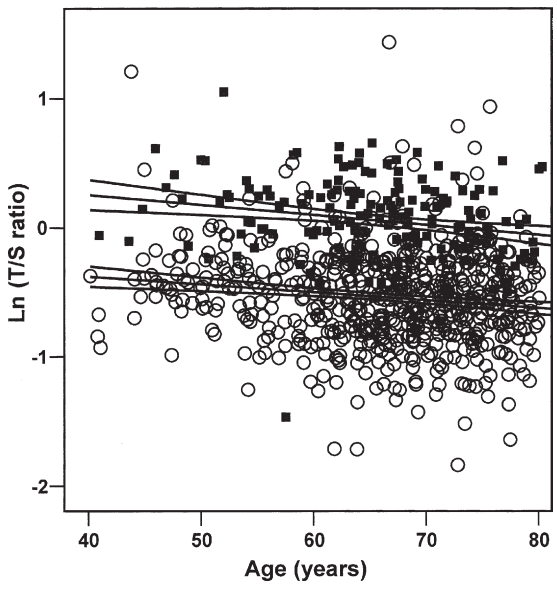

Figure 1 **Telomere Length in Control Patients and CHF Patients**

Telomere length, expressed as the natural log (Ln) of the telomere to single reference gene (T/S) ratio, is plotted as a function of age. The median telomere length was 0.64 (interquartile range [IQR] 0.47 to 0.88) in chronic heart failure (CHF) patients compared with 1.05 (IQR 0.86 to 1.29) in control patients ($p < 0.001$). Yearly decline of telomere length ratio was 0.0079 ± 0.0022 in CHF patients and 0.0077 ± 0.0026 in control patients (p = NS). Linear regression line and mean 95% confidence interval lines are drawn for CHF patients and control patients. **Solid squares** = controls; **open circles** = CHF.

locations was incrementally associated with reduced telomere length. Indeed, with an increasing number of atherosclerotic manifestations present, telomere length was significantly and gradually shorter (Fig. 3). This remained statistically significant after adjustment for age and gender. In contrast to telomere length, there was no association between chronological age and previous myocardial infarction (correlation coefficient 0.012), stroke (correlation coefficient 0.064), and peripheral vascular disease (correlation coefficient 0.053) in our CHF population.

Telomere length was not related to the primary end point of the MERIT-HF study (all-cause mortality or all-cause hospitalization, time to first event) during 339 ± 99 days of follow up (median 339). However, the primary end point of the MERIT-HF study was mainly driven by all-cause hospitalization, accounting for 92% of the events. The objective component of the primary end point, all-cause mortality, was only reached in 13 patients, representing only 8% of the primary end point. In addition, chronological age also did not predict event-free survival.

Discussion

The principal findings of the present study are that telomere length, as determined in peripheral leukocytes, is highly significantly shorter in patients with CHF and also relates to the severity of the disease. Excluding ischemic CHF patients did not change these findings. In addition, we observed telomere length to be shorter in CHF patients with ischemic compared with nonischemic etiology. Finally, in patients with CHF, the concomitant presence and extent of atherosclerotic disease manifestations was found to associate with even greater shortening of telomeres.

The exact mechanism explaining the relationship between shorter telomere length and CHF or atherosclerosis cannot be deduced from the current study. The main question remains—whether shortening of the telomere is cause or consequence in CHF and atherosclerosis, or whether it is simply an epiphenomenon. In mice in which telomerase was genetically knocked out, telomere shortening in subsequent generations was associated with the development of overt CHF (22). Although there is convincing evidence in humans for a role of telomere length in dyskeratosis congenita, a progressive bone marrow failure

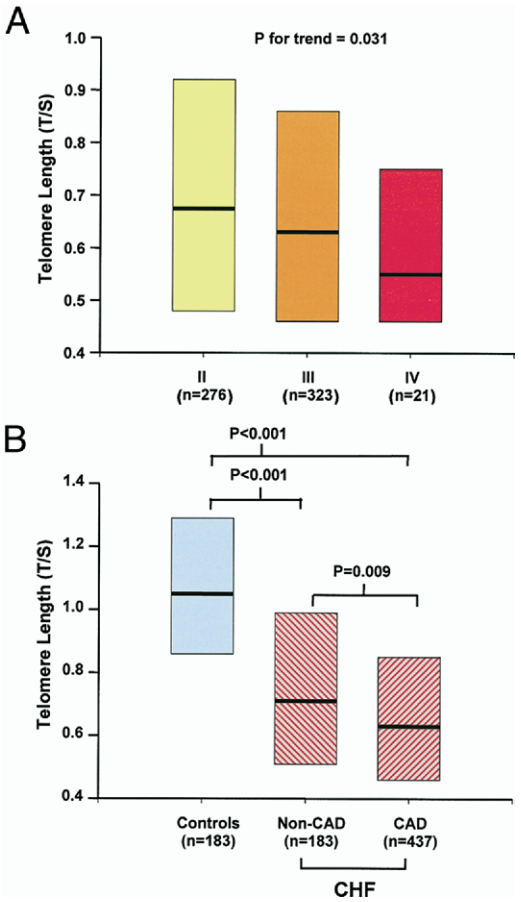

Figure 2 **Relationship Between Severity and Etiology of CHF With Telomere Length**

Median and interquartile range **(box)** of telomere length **(A)** of CHF patients according to the severity of the disease measured by New York Heart Association functional classification; telomere length is incrementally shortened with increased New York Heart Association functional class ($p < 0.05$ for trend). **(B)** Patients with CHF have decreased telomere length compared with healthy control patients ($p < 0.001$). The CHF patients with concomitant coronary artery disease (CAD) have further decreased telomere length ($p < 0.05$). Abbreviations as in Figure 1.

Table 2 Relationships of Age and Telomere Length With Clinical Baseline Characteristics Within Chronic Heart Failure Population (n = 620)

Variable	Telomere (Std β)*	p Value	Telomere Corrected† (Std β)	p Value
Gender	0.058	0.152	0.061	0.126
Age	−0.143	<0.001	−0.144	<0.001
Hypertension	−0.052	0.193	−0.049	0.100
Diabetes mellitus	0.021	0.599	0.019	0.635
Current daily smoker	−0.001	0.988	−0.039	0.340
NYHA functional class	−0.087	0.031	−0.087	0.031
Ischemic etiology CHF	−0.105	0.009	−0.084	0.039
Previous myocardial	−0.082	0.041	−0.071	0.081
Infarction				
Angina	−0.119	0.003	−0.103	0.010
Stroke	−0.104	0.009	−0.099	0.013
Claudication	−0.141	<0.001	−0.130	0.001

*Standardized β (Std β) reflects the change in the dependent variable for 1-SD change in the independent variable. A larger Std β reflects greater strength of the associations. †Adjusted for age and gender.

Abbreviations as in Table 1.

syndrome (23,24), evidence in humans for a causal role of telomeres in the pathophysiology of CHF and atherosclerosis remains to be established. Indeed, both CHF and atherosclerosis are related to increased oxidative stress and inflammation (13–16,25). Oxidative stress and inflammation are important mediators of telomere attrition (5–7) and could explain the observed association.

We observed a linear yearly loss of telomere length ratio of 0.008, which corresponds to approximately 23 to 26 base pairs of telomere sequence lost per year (5,21), and is in good accordance with attrition rates reported by others using alternative assays (3,11,12,26,27). However, when a decreased telomere length is largely acquired, one might expect an increased yearly telomere attrition rate to be associated with CHF or atherosclerosis compared with control patients. However, we did not observe this. This is in good agreement with a previous study, involving patients with premature (<50 years) myocardial infarction (12), and might suggest an important heritable (3,4) component of telomere length. Indeed, it might explain, at least in part, the strong familial basis of CHF and atherosclerosis (28). Conversely and to an equal degree, the comparable yearly attrition rates between CHF patients and control patients could suggest that shorter telomeres have been acquired earlier in life, or possibly even during intrauterine life, and render a phenotype more susceptible to CHF.

Independent of the reasons for the association between telomere length and disease, the important question remains whether variables other than the classic risk factors may distinguish biological from chronological age and predict the occurrence of cardiovascular events and death. A telomere length measurement fulfils a number of criteria for a robust biomarker of human aging. It changes progressively with chronological age, it varies considerably among individuals, and it is connected to the basic biology of aging both as a trigger of cellular senescence and as an indicator of the balance between oxidative stress and antioxidative defense (29). Currently only 1 retrospective study has suggested that short telomere length is an independent predictor of cardiovascular death in 143 subjects ages 60 years or older. However, the survival curves did not seem to divert before the first year of follow-up, and the survival advantage of having longer telomeres was no longer significant in subjects more than 74 years old (30). In the current study, neither telomere length nor chronological age could predict short-term event-free survival during 339 days of follow-up. However, we have to consider that the primary end point consisted of both death and hospitalization for any cause. Only 13 patients (8% of all events) reached the most objective component of the combined end point, namely all-cause mortality. This might have greatly limited our results, and therefore we are cautious about drawing any conclusions. In other words, our study does not refute the hypothesis that telomere length shows important prognostic value in CHF patients. Clearly, telomere length measurements cannot be proposed as routine clinical assessment as of this moment.

The present study has some other limitations that merit consideration. Telomere length per se is not the only critical aspect of telomere maintenance (1). For example, protection of telomere ends is equally important and involves functioning of telomere associated proteins, such as TRF2 (31). We also did not determine telomerase activity. However, it has been shown recently that not telomerase levels but short telomeres themselves are the primary determinants of causing phenotypes (32). Unfortunately, within the MERIT framework no inflammatory markers are available. Therefore, the potential relationship between telomere length and inflammatory markers could not be assessed. During the

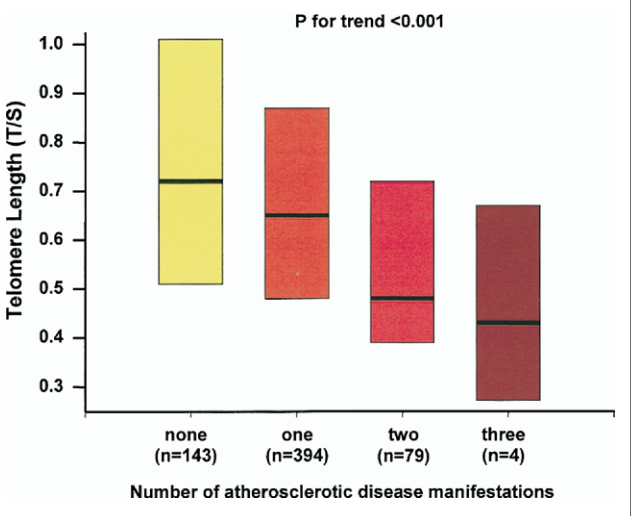

Figure 3 Number of Atherosclerotic Disease Manifestations and Telomere Length in CHF Patients

Median and interquartile range **(box)** of telomere length of CHF patients is gradually decreased according to the number of atherosclerotic disease manifestations: none, 1 (coronary, cerebrovascular, peripheral), 2 (any combination of the previous), or 3. Abbreviations as in Figure 1.

current study, aldosterone blockade in CHF was not common practice (33,34), which might have affected oxidative stress and therefore telomere length. Finally, we reported telomere length as T/S ratio, as determined by a real-time PCR-based method, instead of an "absolute" measure of telomere length such as the (less sensitive) classic Southern blot method on terminal restriction fragments. However, use of real-time PCR-based T/S ratio to quantify telomere length has been confirmed previously to be highly consistent with the Southern blot (5,21). Compared with Southern blot measurements, the real-time PCR approach is more sensitive, more convenient for large-scale studies such as the present one, allows high throughput, requires considerably less DNA, and is less labor intensive (20). In addition, the T/S ratio of our control group was 1.1, the same as that of the control group reported by Broberg et al. (35). Their 93 control subjects, with a median age of 68 years and 19% women (current study, mean age 66 years and 21% women), were comparable with ours.

In conclusion, we found that patients with CHF are characterized by significantly shorter telomeres, and the presence and extent of atherosclerotic disease was accompanied with even shorter telomeres in these patients. Future research should be directed at elucidating the nature of the strong association between telomere length and CHF or atherosclerosis in humans.

Acknowledgment

The authors thank Prof. John Wikstrand, Sahlgrenska University Hospital, Göteborg, Sweden, for his help and support for this MERIT-HF substudy and for critically reviewing the manuscript.

Reprint requests and correspondence: Dr. Pim van der Harst, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9700RB Groningen, the Netherlands. E-mail: p.van.der.harst@thorax.umcg.nl.

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